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The Effects of the Bioactive Constituents of Corydalis Tuber on the Central Nervous System

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ABSTRACT

Corydalis Tuber (CT), a Chinese remedy commonly used for analgesia, was extracted with different solvents and five layers were obtained: methanol, chloroform A, chloroform B, butanol, and water layers. The effects of each layers on CNS were investigated in our present research.

The resutls were as follows:

(1) Acute toxicity: chloroform B layer had the highest toxicity.

(2) Analgesic effect: chloroform B layer and water layer effectively elevated the pain threshold induced by hot plate and 1% acetic acid.

(3) Muscle relaxant effect: water layer had the most significant muscle relaxant effect.

(4) Hypnotic effect: chloroform B layer remarkably prolonged the sleep duration induced by hexobarbital (100 mg/kg, i.p.)

(5) Anticonvulsive effect: chloroform B layer prolonged the latency of clonic convulsion induced by pentylenetetrazole (120 mg/kg, i.p.). Chloroform A layer and water layer prolonged the latency of tonic convulsion induced by picrotoxin (10 mg/kg, s.c.).

The above results showed that the chloroform B layer had the strongest effect on analgesia, hypnosis, and anticonvulsion. Therefore, the chloroform B layer was further chromatographed on silica gel column and eluted with increasing polarization of $CHCl_3$-CH_3OH solution. Each fraction separated from the

chloroform B layer was chromatographed again. Finally, five protoberberine type alkaloids, namely, C-B1 ((−)-tetrahydropalmatine), C-B2 ((+)-corydaline), C-B3 ((±)-tetrahydrocoptisine), C-B4 ((−)-tetrahydrojatrorrhizine), and C-B5 ((±)-palmatine) were isolated and their structures were identified by spectormetric methods.

From the pharmacological studies of the five protoberberine type alkaloids we found that:

(1) Analgesic effect: C-B3 elevated the pain threshold induced by hot plate and 1% acetic acid; C-B4 could only elevate the pain threshold induced by hot plate; all the other fractions had no analgesic effect.

(2) Hypnotic effect: C-B3 prolonged the sleep duration induced by hexobarbital; C-B5 could shorten the latency and prolong the sleep duration induced by hexobarbital; all the other fractions had no hypnotic effect.

INTRODUCTION

With the ever -increasing tension in our modern life, pain syndrome and insomnia become two main complaints leading patients to seek for medical help. All the modern drugs with analgesic and hypnotic effects have their own inevitable side effects.To find a more effective and less toxic drug for the pain syndrome and insomnia is an ever-searching goal of our pharmaco-medical staffs.

Corydalis Tuber was described in Pen Tsao Kang Mu (本草綱目)for "circulatory improvement" and analgesia[1] and also in Lei Kung P'ao Chiu Lun (雷公炮炙論) for "curing headache". Traditional herb physicians used CT for these purposes effectively[2]. Prof. Chen et al. reported that CT was a CNS active herb[3]. The present studies were intended to further investigate its mechanisms by behavior pharmacological methods and try to isolate the pharmacologically active constituents by the phytochemical methods.

MATERIALS AND METHODS

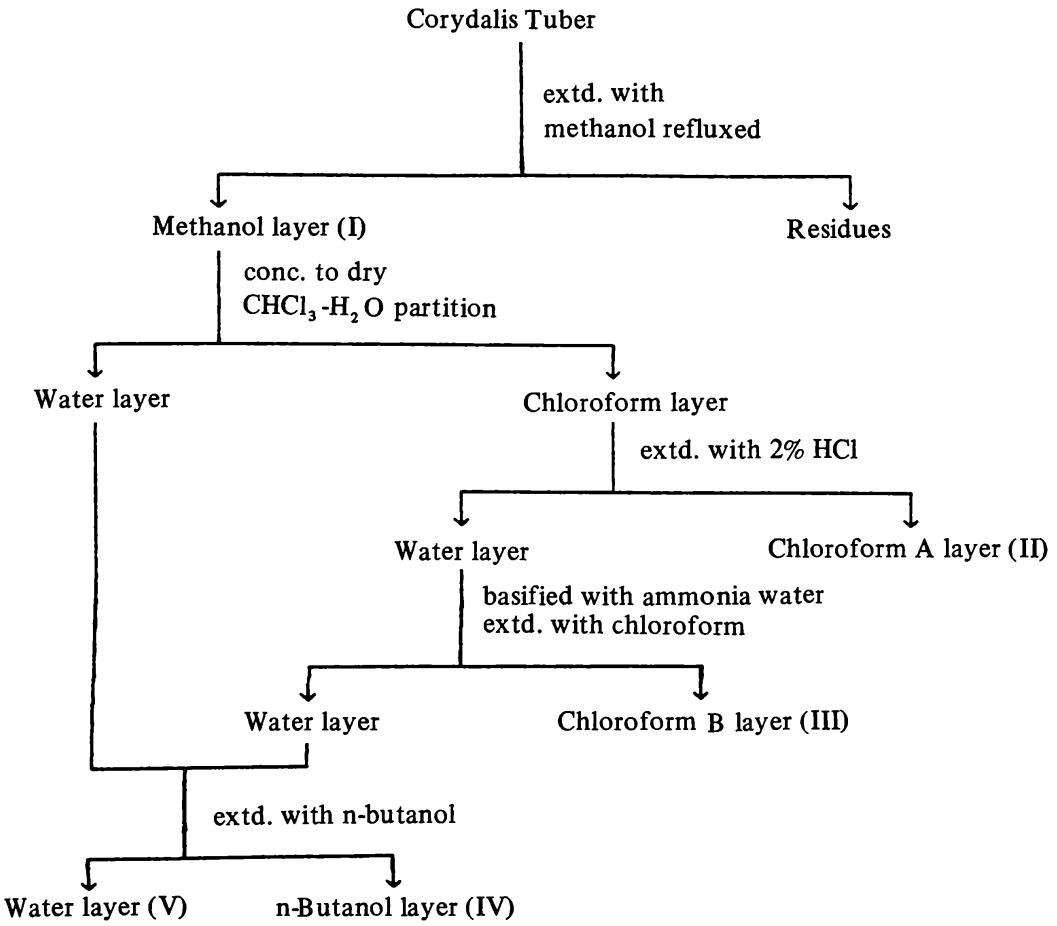
Animals

Male ICR mice, weighing between 18-25 gm, were used in the studies of acute toxicity, analgesic, muscle relaxant, sedative, and anticonvulsive effects, muscle relaxant, sedative, and anticonvulsive effects. The animal were maintained in groups of 5 per cage at a controlled temperature of 22±1°C, humidity of 55±5%, and housed in a metal-wire cage for at least 7 days before each experiment. Solid diets (Sin Lon Co., Taichung) and tap water were provided.

Materials

Corydalis yanhusuo W. T. Wang (Papaveraceae) was used in our experiments.

Melting points were taken on a microhot-stage apparatus and were uncorrected. Optical rotation were measured on a JASCO DIP-4 automatic polarimeter at room temperature. NMR spectra were run on a Bruker AC-250 spectrometer with tetramethylsilane as an internal standard. MS spectra were taken on a JEOL JMX-300 mass spectrometer at 70 eV.



Separation of Corydalis Tuber

In our experiment, five protoberberine-typed alkaloids, namely, C-B1 ((−)-tetrahydropalmatine), C-B2 ((+)-corydaline), C-B3 ((±)-tetrahydrocoptisine), C-B4 ((−)-tetrahydrojatrorrhizine) and C-B5 ((±)-palmatine), were isolated and their structures were identified by spectrometric methods.

Acute toxicity

A sufficient number of animals were used in this test. According to the method of Litchfield and Wilcoxon,[4] the LD_{50}s' (95% confidence limits) were assessed 72 hours after oral administration of each test agent.

Aalgesic effects

Hot plate method: In the experimented group, each test agent (50 mg/kg p.o.) was administered 30 min after mice were placed on a hot plate (55±0.5°C). The latency of heat induced a pain response. Jumping, licking, or withdrawal backpaws were used as the indices and measured at 30 min intervals. In the control group, normal saline was used instead of the test agents.

Acetic acid method: In the experimented group, each test agent (50 mg/kg, p.o.) was administered. One hour later, 1% (v/w) acetic acid (0.1 mL/10 gm) was given intraperitoneally to produce pain. The frequency of writhing reaction was used as the index of pain response and measured at 5 min intervals. In the control group, normal saline was administered.

Muscle relaxant effects

In the experimented group, each test agent (50 mg/kg, p.o.) was administered. The time period of hooking at the iron-wire by backpaws was used as the index of muscle relaxation and measured at 30 min intervals. In the control group, normal saline was administered.

Hypnotic effect

In the experimented group, each test agent (50 mg/kg, p.o.) was administered. One hour later, hexobarbital (100 mg/kg, i.p.) was given to induce sleep. The interval between loss and recovery of righting reflex was used as the index of the hypnotic effect. In the control group, normal saline was used instead.

Effects on CNS stimulant-induced convulsions

In the experimented group, each test agent (50 mg/kg, p.o.) was administered. One hour later, strychnine nitrate (2 mg/kg, i.p.), pentylenetetrazole (120 mg/kg, i.p.), or picrotoxin (10 mg/kg, s.c.) was administered. In the control group, normal saline was used instead. The latency of CNS stimulant induced clonic convulsions. The interval between convulsion and death by gross observation were used as the indices of the effects of each test agent on drug-induced cnvulsions.

Statistical analysis

Results were analyzed statistically by using analysis of the unpaired Student's *t*-test. A p value below 0.05 was required for statistical significance.

RESULTS

Separation of Corydalis Tuber

C-B1 (−)-tetrahydrocoptisine:

colorless needles ($CHCl_3$-MeOH), mp 199-200.5°C.

$[\alpha]_D^{28} = -297.14°$ (c=0.119, $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 5.91 (2H, s, -O-CH_2-O-), 5.94 (2H, s, -O-CH_2-O-), 6.58 (1H, s, H-4), 6.63 (1H, d, J=8.05 Hz, H-12), 6.69 (1H, d, J=8.05 Hz, H-11), 6.72 (1H, s, H-1).

MS (m/z, %): 323 (M^+, 30), 174 (18), 148 (100).

C-B2 (+)-corydaline:

colorless prism (MeOH-ether), mp 138-139.5°C

$[\alpha]_D^{26} = +317.29°$ (c=0.204, $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, d, J=6.84 Hz), 3.87 (12H, s, -OCH_3 × 4), 6.61 (1H, s, H-4), 6.69 (1H, s, H-1), 6.85 (1H, d, J=8.40 Hz, H-12), 6.91 (1H, d, J=8.40 Hz, H-11).

MS (m/z, %): 369 (M^+, 60), 354 (19), 192 (10), 190 (13), 178 (100), 163 (18).

C-B3 (±)-tetrahydropalmatine:

colorless needles ($CHCl_3$-MeOH), mp 146-147°C.

$[\alpha]_D^{28} = 0$ (c=0.175, $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 3.85 (6H, s, -OCH_3 × 2), 3.88 (6H, s, -OCH × 2), 6.62 (1H, s, H-4), 6.73 (1H, s, H-1), 6.79 (1H, d, J=8.34 Hz, H-12), 6.89 (1H, d, J=8.34 Hz, H-11).

MS (m/z, %): 355 (M^+, 100), 354 (70), 190 (33), 164 (100), 149 (75).

C-B4 (−)-tetrahydrojatrorrhizine:

colorless amporphous powder, mp 227-229°C (dec.).

$[\alpha]_D^{25} = -295.35°$ (c=0.175, $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 3.85 (6H, s, $-OCH_3 \times 2$), 3.87 (3H, s, $-OCH_3$), 6.58 (1H, s, H-4), 6.78 (1H, d, J=8.25 Hz, H-12), 6.81 (1H, s, H-1), 6.87 (1H, d, J=8.25 Hz, H-11).

MS (m/z, %): 341 (M^+, 83), 340 (48), 310 (15), 164 (100), 149 (70).

C-B5 (±)-palmatine:

yellow needles ($CHCl_3$-MeOH), mp 234-236°C.

$[\alpha]_D^{28} = 0$ (c=0.164, $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 3.20 (2H, t, J=5.48 Hz, H-5), 3.96, 4.02, 4.08, 4.12 (each 3H, s, $-OCH_3 \times 4$), 5.13 (2H, t, J=5.48 Hz, H-6), 6.92 (1H, s, H-4), 7.15 (1H, s, H-1), 7.26 (1H, s, H-13), 7.88 (1H, d, J=9.33 Hz, H-12), 7.94 (1H, d, J=9.33 Hz, H-11), 10.48 (1H, s, H-8).

MS (m/z, %): 352 (M^+, 34), 351 (21), 149 (21), 148 (100).

The extracted layers of CT

Acute toxicity

Table 1 shows the LD_{50} and 95% confidence limits of various layer of CT.

Table 1. LD_{50} and 95% Confidence Limits of CT in Mice.

Drug	Route	LD_{50} (g/kg)	95% confidence limits (g/Kg)
MeOH ext.	P.O.	5.145	4.49−5.90
$CHCl_3$ A ext.	P.O.	11.00	15.29−7.19
$CHCl_3$ B ext.	P.O.	1.42	1.68−1.20
BuOH ext.	P.O.	3.45	5.11−2.33
H_2O ext.	P.O.	7.70	9.56−6.21

CT: Corydalis Tuber.

Table 2. Analgesic Effect of CT in Mice (Hot Plate Method).

Drug	Time of response (sec) / Time / Dose	0 (min)	30 (min)	60 (min)	90 (min)
Control		5.96±0.78	6.65±0.87	5.39±0.55	6.93±0.79
MeOH ext.	P.O. 50 mg/kg	5.54±0.89	6.68±0.73	10.04±1.02**	6.84±0.98
$CHCl_3$ A ext.	P.O. 50 mg/kg	6.66±0.57	6.35±0.70	9.73±0.78**	8.61±1.16
$CHCl_3$ B ext.	P.O. 50 mg/kg	7.01±0.74	7.74±0.82	12.54±1.37**	9.48±0.73
BuOH ext.	P.O. 50 mg/kg	7.75±0.58	10.71±1.14*	11.80±1.01**	6.66±0.51
H_2O ext.	P.O. 50 mg/kg	7.13±0.46	10.21±0.92**	10.80±1.13**	7.37±0.77

mean±S.E. (n=8)

*P < 0.05, **P < 0.01

P value as compared with the data of 0-min. by Student's *t*-test of group comparison.

Analgesic effects

Hot plate method: As shown in Table 2, both the pain thresholds of the experimented and control groups were elevated at around 60 min ($p < 0.01$). The water layer, elevating pain threshold from 30 min to 60 min, had stronger analgesic effect ($p < 0.01$).

Acetic acid method: As shown in Table 3, all, except the butanol layer, could inhibit the writhing response induced by 1% acetic acid. Chloroform B and water layers (20.4% and 21.8% protection rate respectively) had significant analgesic effect ($p < 0.01$).

Muscle relaxant effect

As shown in Table 4, water layer had effective muscle relaxant effect and strongest muscle relaxant effect.

Hypnotic effect

Table 5 shows that chloroform B layer significantly prolonged the time interval of hexobarbital-induced sleep ($p < 0.001$).

Effects on CNS stimulant-induced convulsions

Five extracted layers had no effect on strychnine nitrate (2 mg/kg, i.p.) induced convulsion as shown in Table 6. It also shows that only chloroform B layer could apparently prolong the

Table 3. Analgesic Effect of CT on the Writhing Symptom of Mice.

Drug	Dose	Total no. of writhing (for 10 min)	Percent protection	Analgesic effect
Control	—	41.1±0.65	—	—
MeOH ext.	P.O. 50 mg/kg	37.0±1.65*	10.0%	+
$CHCl_3$ A ext.	P.O. 50 mg/kg	36.5±1.44*	11.2%	+
$CHCl_3$ B ext.	P.O. 50 mg/kg	32.7±1.93**	20.4%	++
BuOH ext.	P.O. 50 mg/kg	37.4±1.58	9.0%	—
H_2O ext.	P.O. 50 mg/kg	32.1±2.18**	21.8%	++

mean±S.E. (n=8)

*P < 0.05, **P < 0.01

P value as compared with the data of control by Student's *t*-test of group comparison.

Table 4. Effect of CT on Traction Performance in Mice.

Drug	Dose	No. of mice treated	No. of mice which fell within 5 seconds after drug administration			
			30	60	90	120 (min)
Control	—	6	0	0	0	0
MeOH ext.	P.O. 50 mg/kg	6	3	4	1	1
$CHCl_3$ A ext.	P.O. 50 mg/kg	6	1	3	2	1
$CHCl_3$ B ext.	P.O. 50 mg/kg	6	0	2	1	0
BuOH ext.	P.O. 50 mg/kg	6	0	2	2	1
H_2O ext.	P.O. 50 mg/kg	6	3	6	2	0

Table 5. Effect of CT on the Hexobarbital-Induced Hypnosis in Mice.

Drug	Dose	Onset (min)	Sleeping time (min)
Control	—	6.95±0.68	29.23±2.90
MeOH ext.	P.O. 50 mg/kg	4.83±0.23*	38.10±2.62*
$CHCl_3$ A ext.	P.O. 50 mg/kg	8.83±0.59	27.09±1.09
$CHCl_3$ B ext.	P.O. 50 mg/kg	6.82±0.10	64.72±2.13***
BuOH ext.	P.O. 50 mg/kg	5.68±0.55	38.39±3.17
H_2O ext.	P.O. 50 mg/kg	5.38±0.36	42.28±3.70*

mean±S.E. (n=8)
*P < 0.05, **P < 0.01
P value as compared with the data of control by Student's *t*-test of group comparison.

Table 6. Anticonvulsive Effect of CT in Mice.

Induced Drug	Drug (P.O. 50 mg/kg)	O.C. (min)	T.D. (min)	D/N
Strychnine nitrate (2 mg/kg I.P.)	Contrl	3.68±0.41	1.03±0.19	6/6
	MeOH ext.	4.29±0.35	2.36±0.97	5/6
	$CHCl_3$ A ext.	4.55±0.34	2.09±0.89	5/6
	$CHCl_3$ B ext.	2.71±0.07	0.42±0.09	6/6
	BuOH ext.	4.47±0.47	1.98±0.92	6/6
	H_2O ext.	2.88±0.27	2.56±0.74	6/6
Pentylene-tetrazole (120 mg/kg I.P.)	Contrl	0.73±0.05	3.66±0.60	6/6
	MeOH ext.	0.81±0.08	4.43±0.85	5/6
	$CHCl_3$ A ext.	1.10±0.19	4.55±1.09	6/6
	$CHCl_3$ B ext.	1.00±0.05**	7.62±1.57	4/6
	BuOH ext.	0.72±0.03	3.89±0.66	6/6
	H_2O ext.	0.82±0.04	4.17±1.70	6/6
Picrotoxin (10 mg/kg S.C.)	Contrl	6.63±0.59	6.15±0.53	6/6
	MeOH ext.	7.16±0.24	9.39±1.48	6/6
	$CHCl_3$ A ext.	6.95±0.40	9.23±1.20*	6/6
	$CHCl_3$ B ext.	6.07±0.27	7.15±0.48	4/6
	BuOH ext.	5.94±0.25	6.53±1.19	6/6
	H_2O ext.	5.79±0.23	9.81±1.50*	6/6

Mean±S.E. (n=6)
O.C.: Onset to convulsion
T.D.: Time to death
D/N: No. of death/No. of animal tested
**P < 0.01; *P < 0.05
P value as compared with the data of control by Student's *t*-test of group comparison.

latency of pentylenetetrazole (120 mg/kg, i.p.) induced clonic convulsion ($p < 0.01$). And as also shown in Table 6, chloroform A and water layers prolonged the time period between the onset of convulsion and death induced by picrotoxin (10 mg/kg, s.c.) ($p < 0.05$).

Protoberberin type alkaloids extracted from chloroform B layer of CT

Analgesic effects

Hot plate method: As shown in Table 7, 30 min after giving test agent intraperitoneally, C-B3 (tetrahydropalmatine) had the strongest effect to prolong the latency of heat-induced pain response ($p < 0.01$).

Acetic acid method: As shown in Table 8, only C-B3 (tetrahydropalmatine) could effectively reduce the frequency of writhing response induced by 1% acetic acid ($p < 0.01$).

Table 7. Analgesic Effect of Protoberberine Type Alkaloids of Chloroform B Layer in Mice (Hot Plate Method).

Drug	Time of response / Time / Dose / (sec.)	0 (min)	30 (min)	60 (min)	90 (min)
Control	—	9.13±0.54	8.72±0.78	8.22±0.29	8.38±0.59
C-B 1	I.P. 5 mg/kg	7.73±0.37	8.42±0.90	8.05±0.89	7.07±0.75
C-B 2	I.P. 5 mg/kg	8.55±0.58	11.12±1.04	8.50±0.89	8.33±0.72
C-B 3	I.P. 5 mg/kg	7.42±0.36	11.47±0.94**	7.17±0.49	7.95±0.67
C-B 4	I.P. 5 mg/kg	7.23±0.42	9.52±0.89*	7.48±0.50	7.88±1.05
C-B 5	I.P. 5 mg/kg	8.50±0.59	9.45±0.99	8.73±0.87	8.52±0.93

mean±S.E. (n=6)

*P < 0.05, **P < 0.01

P value as compared with the data of 0-min. by Student's *t*-test of group comparison.

Table 8. Analgesic Effect of Protoberberine Type alkaloids of Chloroform B Layer on the Writhing Symptom of Mice.

Drug	Dose	Total no. of writhing (for 10 min)	Percent protection	Analgesic effect
Control	—	33.17±1.19	—	—
C-B 1	I.P. 5 mg/kg	30.33±1.94	8.6%	—
C-B 2	I.P. 5 mg/kg	30.17±1.05	9.0%	—
C-B 3	I.P. 5 mg/kg	27.00±1.29**	18.6%	++
C-B 4	I.P. 5 mg/kg	27.67±2.30	16.6%	—
C-B 5	I.P. 5 mg/kg	28.33±2.04	14.6%	—

mean±S.E. (n=6)

**P < 0.01

P value as compared with the data of control by Student's *t*-test of group comparison.

Table 9. Effect of Protoberberine Type Alkaloids of Chloroform B Layer on the Hexobarbital Induced Hypnosis in Mice.

Drug	Dose	Onset (min)	Sleeping time (min)
Control	—	5.36±0.39	11.63±1.48
C-B 1	I.P. 5 mg/kg	4.83±0.44	11.15±1.80
C-B 2	I.P. 5 mg/kg	4.23±0.44	11.86±2.16
C-B 3	I.P. 5 mg/kg	4.16±0.54	18.27±1.52**
C-B 4	I.P. 5 mg/kg	4.61±0.33	10.30±2.61
C-B 5	I.P. 5 mg/kg	3.42±0.31**	17.88±2.14*

mean±S.E. (n=6)
*P < 0.05, **P < 0.01
P value as compared with the data of control by Student's *t*-test of group comparison.

Hypnotic effect

As shown in Table 9. C-B3 (tetrahydropalmatine) prolonged the sleep time ($p < 0.01$). C-B5 (palmatine) shortened the latency ($p < 0.01$) and prolonged sleeping time ($p < 0.05$).

DISCUSSION

Corydalis Tuber is an effectively analgesic Chinese herb. To find out the active constituents of CT and to further study their effects on CNS, the present study was performed. This would also help to promote the modernization of Chinese herbs. From the studies, we can deduce the following facts: (1) Chloroform B and water layers could elevate the pain threshold; especially, the dl-tetrahydropalmatine, protoberberine type alkaloid, separated and purified from chloroform B layer. These facts imply that dl-tetrahydropalmatine was the primary constituent for analgesia. (2) Chloroform B layer could significantly prolong the sleep period, so could its constituents, dl-tetrahydropalmatine and palmatine. These facts imply that both dl-tetrahydropalmatine and palmatine were the major constituents for sedative effects. (3) Chloroform B layer could prolong the latency of convulsion induced by pentylenetetrazole, a GABA antagonist acted on the mesencephalic reticular formation in CNS.[5] Chloroform A and water layers could prolong the time period between tonic convulsion and death induced by picrotoxin, a GABA antagonist acted on brain stem to block presynaptic inhibition.[6] Each extracted layer had no effect on convulsion induced by strychnine nitrate, a glycine antagonist for blocking postsynaptic inhibition. These facts imply that the anticonvulsive effect of CT may be at the CNS, not at the spinal cord. (4) Water layer could markedly induce muscle relaxation. This fact imply tht the major constituent to induce muscle relaxation may be in the water soluble portion. According to M.T. Hsieh, et al., muscle relaxant effect has concerned with the inceasing activities of GABAergic system[7]. These facts may support the probability that the anticonvulsant effect of CT had to do with increasing GABAergic activities.

To sum up, in crude extracts of CT, chloroform B layer had strong analgesic, sedative, and anticonvulsant effects though it had high toxicities. Besides, the dl-tetrahydropalmatine obtained from chloroform B layer also had marked sedative and hypnotic effects. Modifying

their structures, enhancing their potancies and reducing their toxicities would deserve further studies.

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REFERENCES

1. 明，李時珍，本草網目，文光圖書公司，p 467 (1966).
2. 中山醫學院編：漢藥之臨床應用，p 262，醫齒藥出版，東京(1979).
3. H. Y. Yang, Y. P. Chen and C. F. Chen, Chin. Med. J., **40,** 193 (1987).
4. J. T. Litchfield and F. Wilcoxon, J. Pharmacol. Exp. Ther., **96,** 99 (1949).
5. F. Velasco, M. Velasco and R. Romo, Electroenephalogr. Clin. Neurophysiol., **56**, 96 (1983).
6. G. A. R. Johnston, Neuropharmacology of amino and inhibitory transmitters. In,The Pharmacological Basis of Therapeutics, 6th ed. (A. G. Gilman, L. S. Goodman, A. Gilman, eds) Macmillan Publishing Co., Inc. New York, 586-587 (1980).
7. M. T. Hsieh, T. Shibuya and H. C. Chen, Naunyn-Schmiedeberg's Arch. Pharmacol., **304,** 111 (1987).

中 文 摘 要

延胡索有效成分對於中樞神經系統之影響

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延胡索爲中藥常用之鎮痛劑，本研究將延胡索以不同溶媒萃取，分別得到甲醇層，氯仿 A 層，氯仿 B 層，丁醇層及水層，探討其對中樞神經系統之影響。

各層之藥理實驗結果如下：

(1)急性毒性試驗：以氯仿 B 層毒性最大。

(2)鎮痛試驗：氯仿 B 層及水層以熱板法或醋酸法對痛閾值提高最顯著。

(3)肌肉鬆弛作用：水層具有肌肉鬆弛作用最顯著。

(4)睡眠作用：氯仿 B 層對 hexobarbital (100 mg/kg I.P.)誘發小白鼠睡眠時間之延長最顯著。

(5)痙攣作用：氯仿 B 層能延長由 pentylenetetrazole 引起之痙攣發作時間；氯仿 A 層及水層則對 picrotoxin 引起的強直性痙攣有延長作用。

由上述結果顯示，氯仿 B 層具鎮痛，睡眠，抗痙攣等作用，且作用最強，因此，本研究再將氯仿 B 層利用矽膠柱層分離，以氯仿-甲醇極性漸增方式沖提，各 fraction 再分別進行層析分離，得到五個 protoberberine type 生物鹼，利用光譜分析及標準品比對證實各化合物依序爲：C-B1 (−)-tetrahydrocoptisine)，C-B2 ((+)-corydaline)，C-B3 (±)-tetrahydropalmatine)，C-B4 ((−)-tetrahydrojatrorrhizine)和 C-B5 ((±)-palmatine)，各成分藥理實驗結果如下：

(1)鎮痛試驗：C-B3 對熱板法及醋酸法皆有增加痛閾值作用；C-B4 則僅對熱板法有效；而餘三者無此鎮痛效果。

(2)睡眠作用：C-B3 能延長由 hexobarbital 誘發小白鼠睡眠之睡眠時間；C-B5 能縮短其 onset 及延長睡眠時間；餘者無此作用。